THE INFLUENCE OF VITAMIN E SUPPLEMENTATION
ON THE OXIDATIVE STATUS OF RAT LIVER

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Abstract - We tested to see if the additional intake of vitamin E in the form of α-tocopheryl-succinate would improve liver antioxidative protection. Thus, we studied the tissue oxidative status in rats supplemented by two doses of the antioxidant over a four week period of time. Our results confirmed that the additional intake of vitamin E decreased the liver lipid peroxidation level and SOD activity level and preserved its vitamin C content. However, the hydrogen peroxide content and catalase activity remained unchanged, probably due to the mechanism of vitamin E liver metabolism.

Keywords: Vitamin E, α-tocopheryl-succinate, liver, antioxidative protection, rats.

INTRODUCTION

The term vitamin E was introduced by Evans and Bishop to describe a dietary factor important for rat reproduction (Evans and Bishop, 1922). Natural vitamin E includes two close classes of fat-soluble compounds, the tocopherols and tocotrienols, each represented by four analogs: α, β , γ, and δ (Ricciarelli et al., 2001).

The antioxidant properties of vitamin E are well known and documented (Packer *et al.*, 2001). The membrane vitamin E is regenerated by vitamin C: one-electron oxidation of the α-tocopherol phenol group generates a phenoxyl radical on the head group, which then migrates to the cytoplasm leaflet of the lipid bilayer and reacts with ascorbate to become re-reduced (May et al., 1998). However, the literature data of the influence of vitamin E on vitamin C metabolism is still very obscure.

Although the antioxidant property of these molecules is similar, distinct biological effects can be explained at the molecular level. This specificity lies in the selective retention of α-tocopherol through the liver α-tocopherol transfer protein, as well as in the preferential interactions of some of the compounds with molecular components of the cells.

Having all this in mind, our aim was to study the influence of vitamin E in the form of α-tocopheryl-succinate on the antioxidative status of rats supplemented by it for four weeks. We determined the activities of copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD) and catalase, the hydrogen peroxide concentration, the level of lipid peroxidation and the total vitamin C content in the liver of experimental animals, as well as the serum vitamin C concentration.

MATERIALS AND METHODS

Design of experiment

Male rats of the Wistar strain (*Rattus norvegicus*) weighing 200±45 g were used for the experiments. The animals were acclimated to 22±1°C and maintained under conditions of 12-h periods of light and dark, with free access to tap water and commercial rat food.

The rats were divided into three groups, each consisting of six animals. The first group was the control group, the second and the third groups were animals supplemented with a low and high dose of vitamin E in the form of α-tocopheryl-succinate.

Vitamin E supplementation

The vitamin E doses were 0.8 mg and 8 mg per kg of rat body weight daily (referred to as the low and high dose, respectively).

The α-tocopheryl-succinate was dissolved in 0.5 ml of ethanol, and then mixed in 1 l of tap water. In this way, the final share of ethanol was limited to 0.5‰ of the water volume, in order to prevent its influence on the animals.

According to our previous experiment performed on a group of six animals during four weeks, the average consumption of water in the rats was 240±5 ml of tap water/kg body weight per day. Therefore, both doses of vitamin E were administered every day dissolved in an appropriate volume of tap water to the appropriate group of animals. This supplementation was permanent throughout the four weeks. During the entire experiment, the control group of rats drank tap water with the same quantity of ethanol and no α-tocopheryl-succinate.

Sample preparation

At the end of the vitamin E supplementation period the animals were killed by decapitation with a Harvard guillotine without anesthesia, as recommended by the Local Ethical Committee. After decapitation, the livers were removed and the blood collected.

The tissue was excised and divided into two equal portions. One portion was homogenized in 25 mM phosphate buffered saline (PBS) pH 7.0 and centrifuged at 9000 x g in a semi-preparative Sorvall Super T21 centrifuge for 20 min at 4°C. The supernatants were used for determination of the catalase, CuZnSOD and MnSOD activities, as well as for the measurement of the concentration of hydrogen peroxide and total lipid hydroperoxides (the level of lipid peroxidation). The other portion of livers and blood serums were used for determination of vitamin C concentration by using a similar procedure, except that 6% trichloroacetic acid (TCA) was used instead of PBS.

Methods

Total vitamin C content was determined by the method of Roe and Kuether (1943), and calculated against its standard curve absorption values.

Total superoxide dismutase activity was determined in the tissue PBS samples by the adrenaline method of Misra and Fridovich (1972), using potassium cyanide as a CuZnSOD inhibitor thus allowing differential calculation of MnSOD activity (Weisiger and Fridovich, 1973).

Catalase activity was measured in the tissue PBS samples by the method of (Beutler, 1982), which is based on the rate of H_2O_2 degradation by the action of catalase contained in the examined samples.

Measurement of the total lipid hydroperoxides and H_2O_2 content were both based on the ferrous ion oxidation by xylenol orange (FOX) assay (Gupta, 1973; Wolf, 1994). Two versions of FOX assays are described in the literature, FOX-1 and FOX-2 (Banerjee et al., 2003). The concentration of H_2O_2 was measured by the FOX-1 assay (Gay and Gebicki, 2000), and calculated against the hydrogen peroxide standard curve absorption values. The concentration of lipid hydroperoxides was measured by the FOX-2 assay (Jiang et al., 1991), with the level of lipid peroxidation in samples expressed as a percent of the lipid peroxidation level of the control animal group.

Statistical analysis

The data are expressed as means ± SEM. The values obtained from rats without vitamin E supplementation were used as a control. One-way ANOVA

was undertaken for the multiple range comparison. Significant differences among the groups were determined by the Tukey test. The probability of significance of differences was set at $P < 0.05$.

RESULTS

In the serum and liver of rats fed with vitamin E, the endogenous concentration of ascorbate decreases in a dose-dependent fashion. In addition, the level of lipid peroxidation in the liver was also dose-dependently reduced under the influence of this antioxidant (Fig. 1).

In the liver of rats fed with vitamin E, the activities of both CuZnSOD and MnSOD were decreased (Fig. 2). However, the hydrogen peroxide concentration in the tissue remained unchanged in relation to the control level, as well as the activity of catalase (Fig. 3).

DISCUSSION

Based on our results, we can conclude that vitamin E causes the decrease of the lipid peroxidation level in the liver. It also lowers liver MnSOD activity, probably due to its especially large accumulation in the inner mitochondrial membranes (Ibrahim et al.,
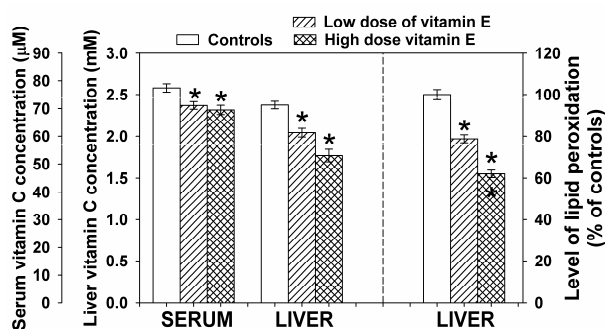

Fig. 1. The influence of low and high doses of vitamin E supplementation on the endogenous concentration of vitamin C in rat serum and liver (left), and the level of liver lipid peroxidation (right). Statistically significant differences ($P < 0.05$) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between two doses of the vitamin E are marked with an asterisk inside the columns.
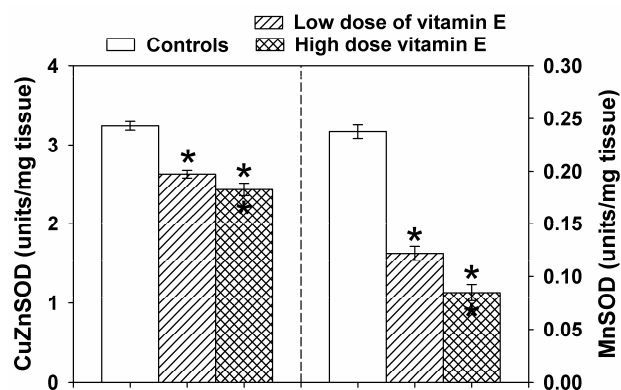

Fig. 2. The influence of low and high doses of vitamin E supplementation on the activities of CuZnSOD (left) and MnSOD (right) in rat liver. Statistically significant differences ($P < 0.05$) relative to the control are marked with an asterisk above the columns, while statistically significant differences between the two doses of vitamin E are marked with an asterisk inside the columns.
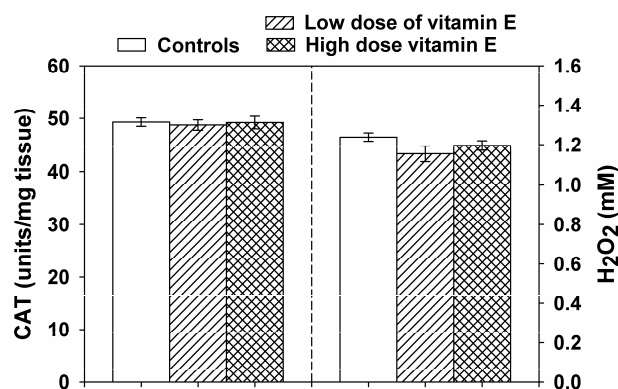

Fig. 3. The influence of low and high doses of vitamin E supplementation on the activity of catalase (left) and the concentration of H_2O_2 (right) in rat liver.

2000). Acting there together with the coenzyme Q10 (Dallner and Sindelar, 2000), vitamin E protects against respiratory oxidative stress (Ham and Liebler, 1995).

Through the prevention of the "oxidative stress transfer" from mitochondria to cytoplasm (Tanaka et al., 1997), vitamin E is probably preserves the ascorbate content in the cell. As can be seen from our results, vitamin E decreases the concentration of vitamin C in both the serum and the liver,

indicating that its supplementation has a negative effects on liver ascorbate synthesis (Moreau and Dabrowski, 2003); an increased serum ascorbate level in rats poisoned by cadmium could be lowered by the simultaneous vitamin E pretreatment (Ognjanovic et al., 2003).

Our results show that vitamin E also lowers the liver CuZnSOD activity level, which is in agreement with literature data. This suggests that its supplementation decreases total liver (Muller and Pallauf, 2003), heart (Prasad and Kalra, 1989) and serum (Mantha et al., 1993) SOD activities.

However, regardless of the decrease in liver SOD activity, the hydrogen peroxide content (as the subsequent product of SOD activity), and catalase activity both remain unchanged in the livers of vitamin E-fed rats;. Vitamin E is known to be metabolized similarly to xenobiotics: it is initially ω-oxidized in the liver by certain members of the cytochrome P450 family enzymes (Birringer et al., 2001). It undergoes several rounds of β-oxidation, and is then conjugated and excreted (Traber, 2007). It can be appreciated that the P450 reaction cycle produces some forms of reactive oxygen species, namely superoxide and peroxide (Lewis, 2002). Protonation of the peroxide yields H_2O_2, which can maintain its liver concentration unchanged despite the decrease in SOD activity.

It should be emphasized that the ratio of the applied doses of vitamin E (i.e. 1:10) differs far more than their respective effects on the examined parameters. The explanation probably lies in the mechanisms of vitamin E absorption, tissue accumulation and metabolism. For example, it is known that the α-tocopherol capacity of blood is very limited: regardless of the intensity or duration of supplementation. The concentration of this antioxidant in the circulation cannot exceed more than two or three times its physiological value (Dimitrov et al., 1991). This is apparently not due to limited absorption, since α-tocopherol is absorbed at a constant fractional rate with increasing dose (Traber et al., 1998). Thus, the underlying reasons may include variations in α-tocopherol transfer

protein activity, metabolic rate, lipid content and composition, the status of other micronutrients that recycle α-tocopherol and environmental conditions. Moreover, newly absorbed vitamin E molecules need to replace old ones in plasma lipoproteins, which may be the limiting step in the overall incorporation (Burton et al., 1998).

In conclusion, our results confirm that the additional intake of vitamin E improves the liver antioxidative defense in a dose-dependent manner. The discrepancy between the ratio of the applied doses and their respective effects on the examined parameters is probably the consequence of vitamin E absorption, tissue accumulation and metabolism.

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ПРОМЕНЕ ОКСИДАТИВНОГ СТАТУСА ЈЕТРЕ ПАЦОВА ПРЕХРАЊИВАНИХ ВИТАМИНОМ Е

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Испитиван је ефекат прехране пацова са две дозе витамина Е у форми алфа-токоферил-сукцината на оксидативни статус јетре. Одређивани су активност бакар цинк супероксид дисмутазе, манган супероксид дисмутазе и каталазе, ниво липидне пероксидације и концентрација водоник пероксида и укупног витамина Ц у јетри експерименталних животиња, као и концентрација витамина Ц у серуму. Добијени резултати указују да витамин Е повећава антиоксидативну заштиту у јетри пацова, на начин који указује на специфичан значај механизама његове апсорпције, ткивне акумулације и метаболизма.